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testable predictions. The time dependent vibrational mean square displacement of an amino acid is predicted to be subdiffusive. The thermal variance in the instantaneous distance between amino acids is shown to grow as a power law of the equilibrium distance. Mean first passage time analysis is offered as a practical tool that may aid in the identification of amino acid pairs involved in large conformational changes.

1222-Pos Board B132
Dynamical Alignment of Solution Phase Proteins for Structural Measurements
Deepu K. George, Rohit Singh, Chejin Bae, Byungwook Ahn, Kwang Oh, Andrea Markelz.
For a variety of structural measurements, aligned molecular systems are needed. However, crystallization conditions cannot always be found, or the required crystal size is out of reach for standard growth procedures. In this project, we have devised a method to create partially aligned solution phase protein systems utilizing the large dipole moment for proteins. We have developed a solution cell capable of applying very large DC and AC electric field to protein solution in order to align them dynamically to the electric field of the probing beam. Narrow channels 100 μm wide and 50 μm in thickness are fabricated on an IR grade quartz substrate with SU-8 photo resist using photo lithographic methods. Two separate sets of electrodes are formed on the side walls on these channels by angular metal deposition. A second quartz substrate UV bonded to the top surface of the SU-8 walls forms the lid. Our cell has high (>85%) transmission in the far infrared and visible region and at the same time the electrodes on the side walls provide an electric field of the order of 10s of kV/cm. We first demonstrate the cell's ability to modulate alignment using visible transmission of liquid crystals. Further, by dynamically aligning the protein molecules in the solution to an alternating electric field and by locking in to that frequency we can enhance structurally related signals by several orders of magnitude and also distinguish between the different structural modes. This work is supported by NSF MRI^2 grant DBI2959989.

1223-Pos Board B133
Infrared Spectra of the Azide Asymmetric Stretching Band in a Model Compound, a Peptide Containing Beta-Azido Alanine, and in Carbohydrates
Marta P. Wolfshorndl, Stephen Griffiths, Casey H. Londergan.
Infrared spectra of the model compound 5-azido,1-pentanoic acid were used to characterize the behavior of the asymmetric azide stretching peak in different solvents. The frequency and width of the peak changed depending on the solvent. The greatest change was seen between all non-aqueous azide bands and the band in water, which was blue shifted substantially from the nonaqueous peaks. Spectra were collected in mixed water/organic solvent solutions, sugar/water solutions, and under changing temperatures to determine the behavior of the azide peak. A model alanine-repeat peptide with an azide-labeled amino acid was synthesized, and temperature-dependent IR experiments of the aqueous peptide were used to assess the sensitivity of the azide peak to peptide folding. The possible application of site specific azide labeled carbohydrates has also been explored.

1224-Pos Board B134
Asymmetry in Rabbit Muscle Creatine Kinase from the Perspective of Active Site Cysteines
Kevin W. Hoffman, Connor G. Bischak, David M. Snead, Stephen Griffiths, Christopher Reynoso, Casey H. Londergan.
Creatine Kinase (CK) has been the subject of extensive long-term study because of both its biological importance and its peculiar structural behavior. In vertebrates, the enzyme is functional as a dimer. The apo crystal structure indicates a symmetric dimer, but more recent studies of transition state analog complexes have shown that CK selectively binds substrate with only one monomer, suggesting a functional asymmetry in the dimer. All creatine and arginine kinases display a highly conserved single cysteine residue in the active site whose role is unclear. Infrared and 13C-NMR spectroscopy are used here to study the environment around the cyanylated Cysteine 283 residue of solution-phase rabbit muscle creatine kinase in both the all-apo and transition state analog forms of the enzyme, and Raman spectroscopy is used to observe the environment of the unmodified thiol group at the same positions. Each measurement exhibits dynamic asymmetry between the two Cys283 residues in the dimer, suggesting that the structure of rabbit muscle creatine kinase is inherently asymmetric in solution regardless of induced fit due to substrate binding. The possible extension of these novel spectroscopic approaches to other CKs, arginine kinases and other enzyme active sites will be discussed.

1225-Pos Board B135
Cyanylated Cysteine used to Characterize Binding Interfaces of Calcium Binding Proteins
Shannon R. Dalton, Matthew S. Puretz, Casey H. Londergan, Sara Linse.
Calmodulin is completely conserved in eukaryotes and participates in signaling by binding many different target proteins. Most of these binding interactions have not been characterized due partly to calmodulin's inherent flexibility, both in structure and binding mode. Infrared absorption spectra of carefully placed side-chain labels can report on dynamic protein structure in cases where more conventional techniques fail. Cyanylated cysteine residues have been placed in locations of interest on a calmodulin-bound target peptide with a known calmodulin-peptide structure with the goal of demonstrating the sensitivity of cyanylated cysteine to different locations of the binding interface. Subsequently, the vibrational labels will be moved onto calmodulin and used to distinguish between different binding geometries of calmodulin with uncharacterized natural targets. Placement of the cyanylated cysteine probe inside the hydrophobic interior of a collapsed calcium-binding protein will also be discussed.

1226-Pos Board B136
Luminescence from Extrinsic and Intrinsic Probes in Solid State Amorphous Human Serum Albumin Demonstrates Solvent-Protein Slaving
Andrew R. Draganski, Minhazur Rahman, Richard D. Ludescher.
The physical properties of amorphous biomolecules are important to the stability of low-moisture foods and pharmaceuticals. Freeze-dried proteins are often stabilized via inclusion of excipients. The effect on protein dynamics of substitution of surface water with sugars is unclear. To explore this question, we have conducted luminescence studies on human serum albumin (HSA) in the dry amorphous state using both extrinsic probes and intrinsic tryptophan. Phosphorescence is an ideal approach, as the long-lived triplet state of molecular probes is sensitive to the long time-scale motions of dry proteins. HSA binds luminescence probes that report on the protein's surface; it contains a single, buried tryptophan that reports on the interior of the protein. Amorphous protein-sugar films were prepared by spreading concentrated solutions of sugar + HSA with bound probe onto quartz slides, followed by rapid drying and extensive desiccation. We have bound the water-sensitive probe pyranine to HSA and measured its fluorescence spectra to extract information on the amount of the water in the protein's hydration shell. We have also made dry films from sugars + HSA bound with erythrosin B or vanillin and collected these probe's phosphorescence decays as a function of temperature. These measurements are compared to those of HSA's buried tryptophan residue. Intensity decays were fit with multi-exponential functions and the rates of non-radiative decay (kNR) were calculated from the average lifetime; kNR is dependent on the microviscosity of the site and is thus a measure of local molecular mobility. The degree to which the fits are non-exponential reveals dynamic heterogeneity experienced by the probe. Analysis of kNR Arrhenius plots reveal relations between the mobility in the bulk solid solvent with that at the surface and in the interior of the protein.

1227-Pos Board B137
Fibrin Fibers Exhibit Two Distinct Temporal Regimes of Recoil Dynamics
Nathan Hudson, Hans Peng, E. Timothy O'Brien, Susan Lord, Richard Superfine, Michael Falvo.
Fibrin fibers form the mechanical backbone of blood clots, stemming the flow of blood at the sights of vascular injury. The heart pumps blood at approximately 1Hz, requiring fibers within the vasculature to recover elastically within a second, or risk clot failure. Recent work on fibrin fiber mechanics showed that fibers are soft in stretching (1-10 MPa modulus), reversibly stretch to up to three times their original length and strain stiffen above 100% strain; however, fibrin fiber dynamics has received less attention. Using a combined AFM/Optical fluorescent microscope we have probed the recoil dynamics of fibrin fibers on the millisecond timescale. Fluorescently labeled fibers were suspended between channels and then stretched from the center with an AFM tip. Fibers were allowed to slip off the tip and fiber relaxation was measured optically at 3-11 ms time intervals. The data indicate two temporal regimes of fibrin dynamics: A fast initial regime ($\leq$1ms) where the fiber snaps back into a loose conformation, and a slow regime on the order of 10's of ms, where the fiber regains a taut conformation. The two timescales of fiber relaxation indicate two different mechanisms. We find that the timescale of the slow regime is directly related to the strain of the fiber at the point of release. The fast timescale is likely governed by the entropic recoil of unstructured polypeptide chains, while the second timescale is likely governed by either protein folding or a macroscopic re-ordering of the fiber substructure. Further experiments will differentiate between these mechanisms, and the implications of these two regimes of fiber recovery will be discussed.